The Oral Microflora in Health and Disease

Opportunities for collaboration

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Outline

• Brief introduction to oral microflora
• Some of my work resulting from an SOD/SON collaboration
• Personal examples of other previous SOD/SON collaborations
• Examples of potential future collaborations
Human Microflora

• Based on cell counts, we are 10 parts bacteria and 1 part human

• Based on gene content, we are 150 parts bacteria, and 1 part human
Oral Microflora

- More than 700 species identified
- Composition changes with time and location
- Normal flora likely serves as a barrier to colonization by pathogens

Oral Microflora

Early SOD/SON Collaboration

- Frank Macrina: *Streptococcus mutans* and dental caries
- Cindy Munro: *Streptococcus mutans* and infective endocarditis?
Infective Endocarditis Pathogenesis

- Normal valve
- Transient bacteremia
- Deposition of platelets and fibrin
- Damaged valve
- Disease, valve replacement
- Sterile vegetation
Infective Endocarditis Pathogenesis
Infective Endocarditis Prevention
Munro and Macrina


Streptococcus sanguinis genome sequence


- 2.388 Mb
- ~2300 genes
The Cell Surface

lipoprotein

cell wall

LPXT

The Cell Surface

lipoprotein

cell wall

LPXT
Lipoprotein candidates

- 53 lipoproteins genes
- Made 53 mutants
- The lipoprotein SsaB was found to be essential for virulence
• *S. sanguis* adhesin B

SsaB

- *S. sanguinis* adhesin B
Growth in serum ± 2 \( \mu \text{M Mn} \)

- **SK36**
- **ssaB**

**CFU/ml** vs **Time (h)**

- From 0 to 25 hours, SK36 shows a growth pattern with approximately 10^5 to 10^9 CFU/ml.
- ssaB shows a decline from 10^4 to 10^3 CFU/ml over the same time period.
Growth in serum ± 2 μM Mn

- **SK36**
- **SK36 + Mn**
- **ssaB**

CFU/ml vs. Time (h)
Growth in serum ± 2 μM Mn

- SK36
- SK36 + Mn
- ssaB
- ssaB + Mn
Inhibition of Mn uptake?

<table>
<thead>
<tr>
<th></th>
<th>Saliva</th>
<th>Serum</th>
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<tbody>
<tr>
<td>Mn conc.</td>
<td>36 μM</td>
<td>20 nM</td>
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</table>
SsaB Family

- S. sanguinis
  - ssaA (ATPB)
  - ssaC (IMP)
  - ssaB (Lpp)

- S. mutans
  - sloA (ATPB)
  - sloB (IMP)
  - sloC (Lpp)

- S. parasanguinis
  - fimC (ATPB)
  - fimB (IMP)
  - fimA (Lpp)

- S. gordonii
  - scaC (ATPB)
  - scaB (IMP)
  - scaA (Lpp)

- S. pneumoniae
  - psaB (ATPB)
  - psaC (IMP)
  - psaA (Lpp)

- S. pyogenes
  - mtsA (Lpp)
  - mtsB (ATPB)
  - mtsC (IMP)
Other Diseases

S. pneumoniae
psa locus

- psaB (ATPB)
- psaC (IMP)
- psaA (Lpp)

S. pyogenes
mtsA locus

- mtsA (Lpp)
- mtsB (ATPB)
- mtsC (IMP)


Callahan, J. E., C. L. Munro, and T. Kitten. 2011. The *Streptococcus sanguinis* competence regulon is not required for infective endocarditis virulence in a rabbit model. PLoS ONE 6:e26403.

Other Collaborations

- “Low-tech” microbiology
Equipment and Resources

- Anaerobic jars
- Temperature-controlled microplate reader
- Anoxomat anaerobic system
- 37°C incubator
- Biosafety cabinet
- Thermal cycler
Other Collaborations

• “Low-tech” microbiology
• Asking neglected questions or old questions in new ways
Other Collaborations

Deborah J Jones, PhD, MS, RN
Oral Care and Bacteremia Risk in Mechanically Ventilated Adults

Michelle Frazelle, PhD, RN, CCRN
Healthcare Acquired Infection Risk and Toothbrush Contamination in the ICU

• Bacterial species identification
• Epidemiological molecular strain typing
Other Collaborations

- “Low-tech” microbiology
- Asking neglected questions or old questions in new ways
- Asking questions that couldn’t be asked previously
Other Collaborations

• Example:
  – Are particular communities of oral bacteria associated with health or a particular disease?
    • Approach: Metagenomics
    • Method: “Next-generation” deep sequencing
Metagenomics

HiSeq 2000/1000
One run mode. High output.

|                          | HiSeq 2000 | GS FLX+
<table>
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<tbody>
<tr>
<td>Output (2 × 100 bp)</td>
<td>600 Gb</td>
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<tr>
<td>Run Time</td>
<td>~11 days</td>
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<tr>
<td>Cluster Generation</td>
<td>cBct</td>
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<tr>
<td>Paired-end Reads</td>
<td>6 Billion</td>
<td></td>
</tr>
<tr>
<td>Single Reads</td>
<td>3 Billion</td>
<td></td>
</tr>
<tr>
<td>Maximum Read Length**</td>
<td>2 × 100 bp</td>
<td></td>
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Also Ping Xu, Ph.D., Janina Lewis, Ph.D.
Other Collaborations?

• Alison Montpetit, R.N., Ph.D.
  – Does the oral microflora contribute measurably to the composition of exhaled breath condensate?
  – Could this be useful for diagnostic purposes?
Collaborations Involving Other SOD Researchers Working with Oral Microflora?
Oral Bacteria in Relation to Extra-oral Diseases

- Infective endocarditis
- Periodontal disease as contributing to atherosclerosis and coronary heart disease
Periodontal disease

- Direct infection or action at a distance
- Associated with
  - Premature birth or low birth weight
  - Diabetes
  - Others?

SOD Faculty Working on Periodontal Disease

Harvey Schenkein, D.D.S., Ph.D.

Esra Sahingur, D.D.S., Ph.D.

Janina Lewis, Ph.D.
What we can do for you

• Provide expertise and equipment for pilot studies involving microbiology
  – “Yes, we can do that!”

• Bring you our ideas for the “next big thing” and be open to yours
What you can do for us

• Bring us your ideas and problems
• Include the SOD in research seminar notices?
• Keep your door open for us (as is already the case)

“These [interdisciplinary] partnerships enrich our research and provide wonderful opportunities for the students working with us on these studies.”

Read more
References


